FIG. 1A.

Library-Encoded Peptides (LEPs) from Genomic Sau3A1 Fragments

S10 or HA Epitope Tag

P_lac DBD

Library Plasmids

Amp^R colE1 ori

Target Peptide: KPAKSARSVRSQR or NEAYVHDGPVRSLN

P_lac DBD Linker Trans. Term.

Target Plasmid

Tet^R pACYC ori

FIG. 1B.

Library-encoded peptide

Target peptide

DBD

RNAP

Operator Promoter lacZ or phage genes

Library-encoded peptide

Target peptide

DBD

RNAP

Operator Promoter lacZ or phage genes

GST-13mer-containing extract

FIG. 2

Wash. Elute with
maltose. Analyze by
SDS-PAGE.

13mer target: KPAKSARSVRSQR
27mer Library-encoded peptide:
THTTSQTTLRDPDVYAGARWVTWRVGA

His₆ — LEPB

(In crude E. coli extract)

Ni•NTA
Agarose

Elute with
imidazole

ICS-
Affi-gel

Elute with
amino acids

FIG. 3

FIG. 4

Preparation of Hybridization Probes

Transform E. coli with DBD-peptide expression vector only. → Challenge with virus. Plate survivors → Isolate plasmids of survivors and amplify peptide-encoding regions by PCR to obtain hybridization probes for homooligomers

Selection Protocol

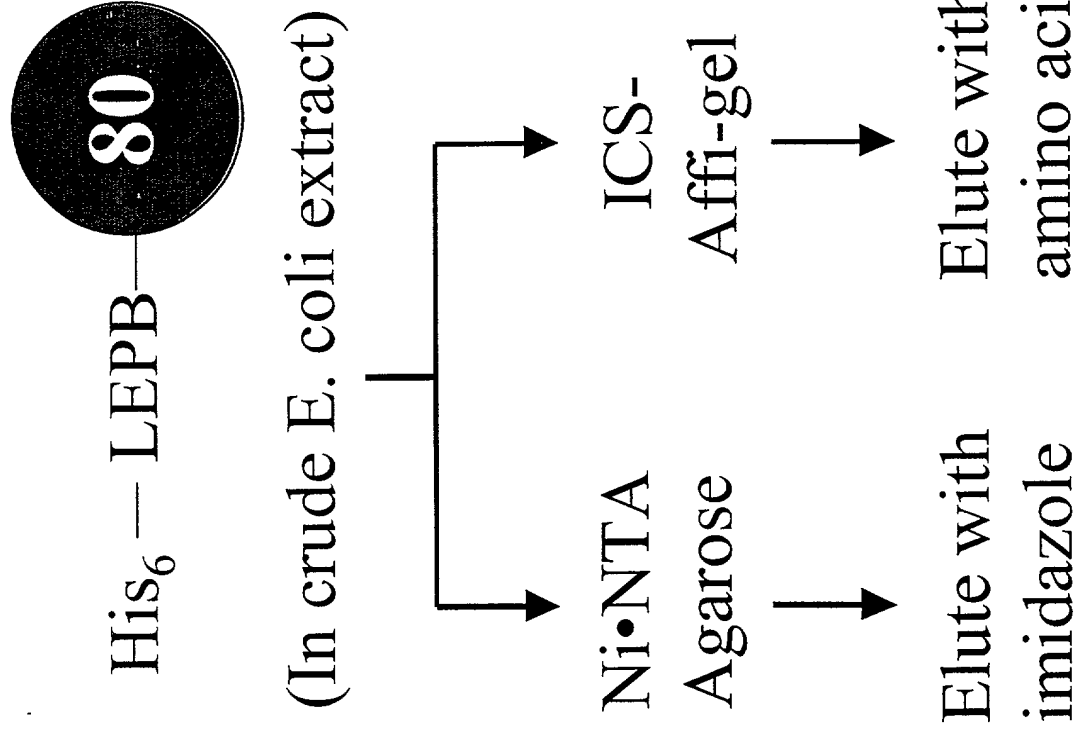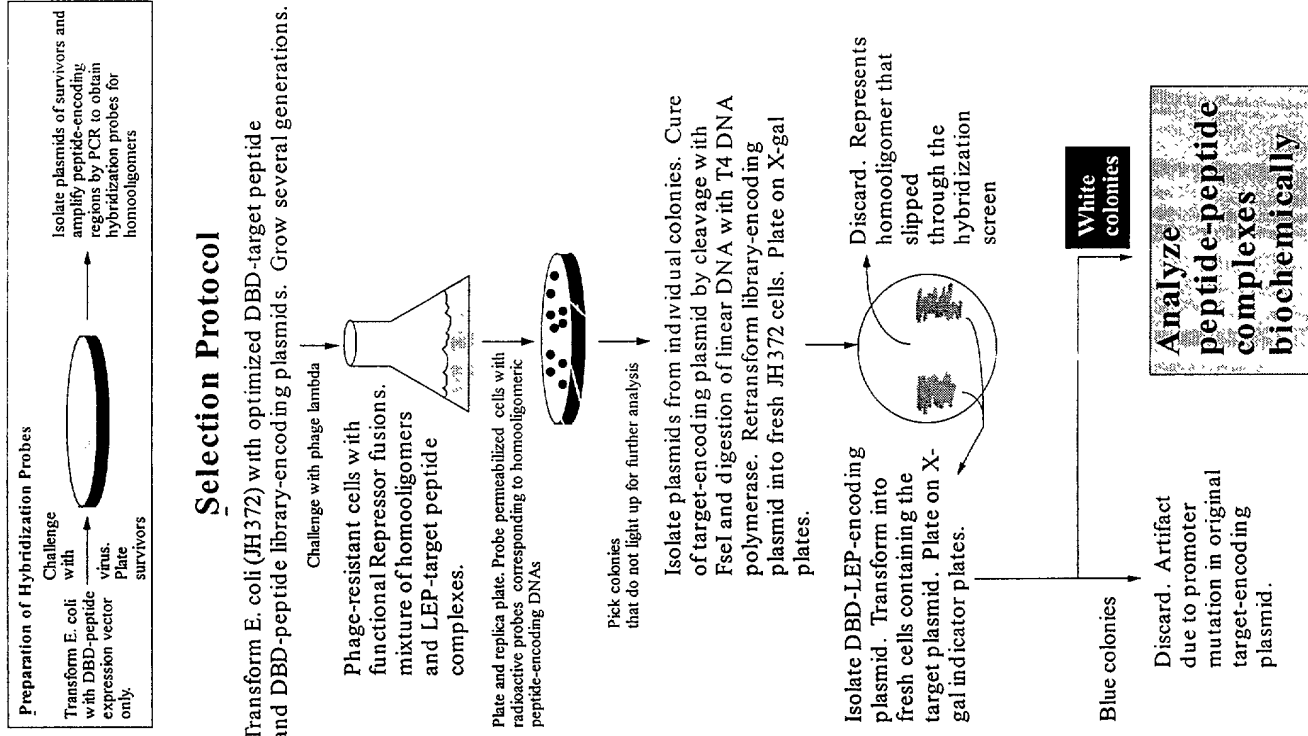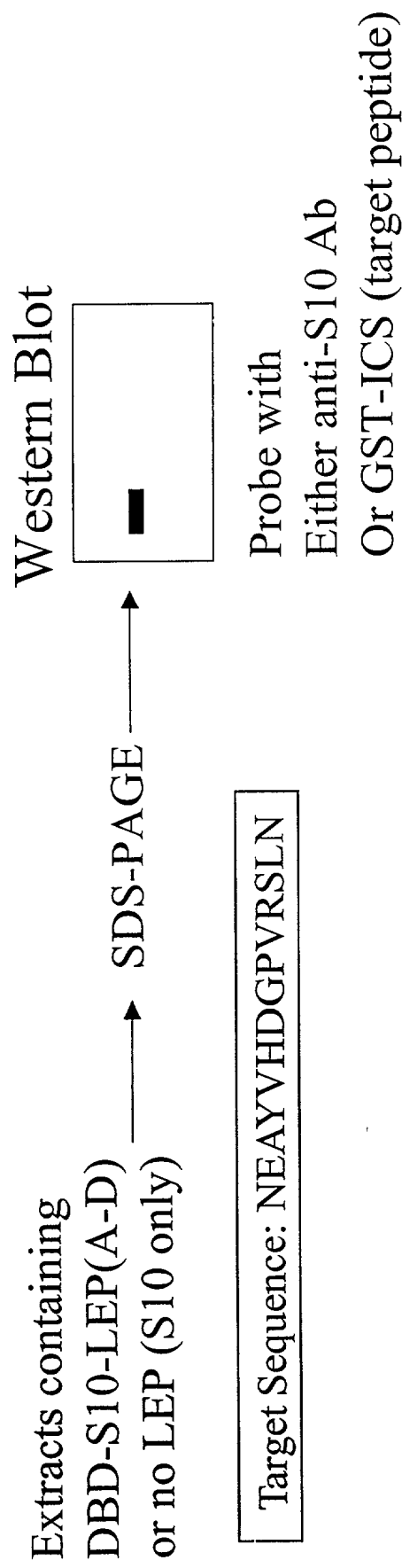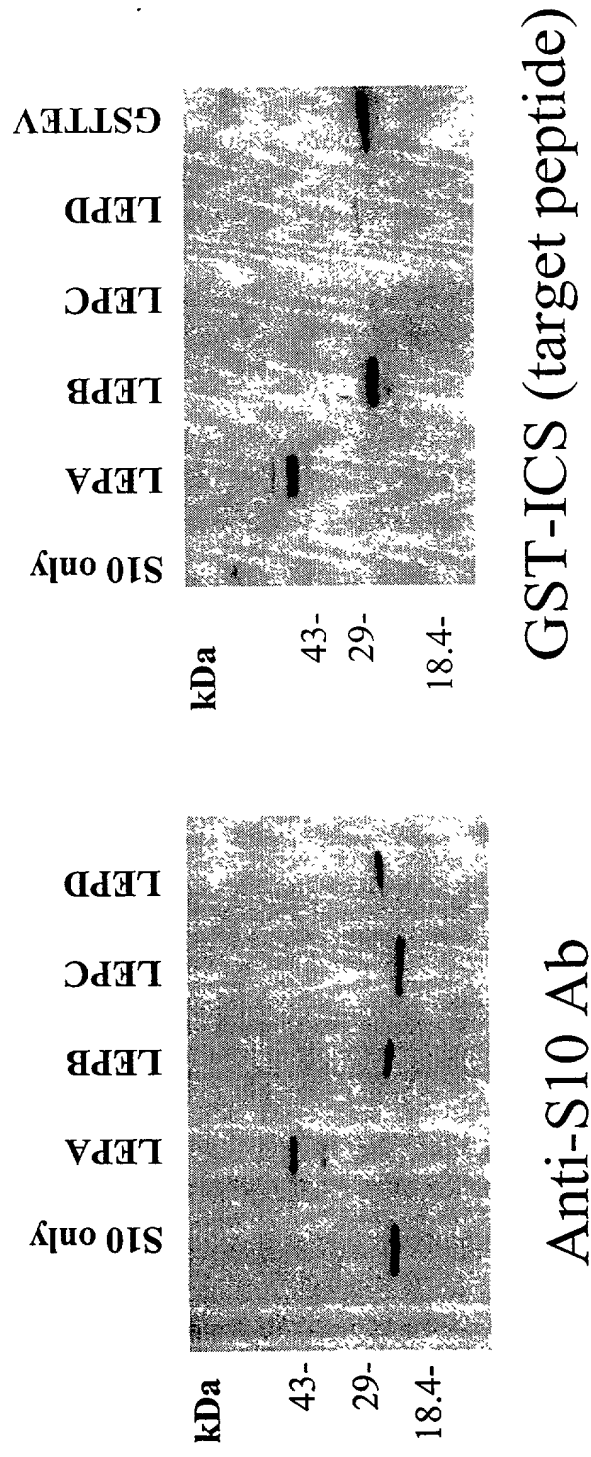Transform E. coli (JH372) with optimized DBD-target peptide and DBD-peptide library-encoding plasmids. Grow several generations.

Challenge with phage lambda

Phage-resistant cells with functional Repressor fusions. mixture of homooligomers and LEP-target peptide complexes.

Plate and replica plate. Probe permeabilized cells with radioactive probes corresponding to homooligomeric peptide-encoding DNAs

Pick colonies that do not light up for further analysis

Isolate plasmids from individual colonies. Cure of target-encoding plasmid by cleavage with FseI and digestion of linear DNA with T4 DNA polymerase. Retransform library-encoding plasmid into fresh JH372 cells. Plate on X-gal plates.

Discard. Represents homooligomer that slipped through the hybridization screen

Isolate DBD-LEP-encoding plasmid. Transform into fresh cells containing the target plasmid. Plate on X-gal indicator plates.

Blue colonies

Discard. Artifact due to promoter mutation in original target-encoding plasmid.

White colonies

Analyze peptide-peptide complexes biochemically

Western Blot

Extracts containing
DBD-S10-LEP(A-D)
or no LEP (S10 only)

$\longrightarrow$ SDS-PAGE $\longrightarrow$

Probe with
Either anti-S10 Ab
Or GST-ICS (target peptide)

Target Sequence: NEAYVHDGPVRSLN

GST-ICS (target peptide)

Anti-S10 Ab

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9